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Tcxicity of Mercuric Chloride to the Developing Rat Kidney. 2. Effect of Increased Dosages on Renal Function in Suckling Pups

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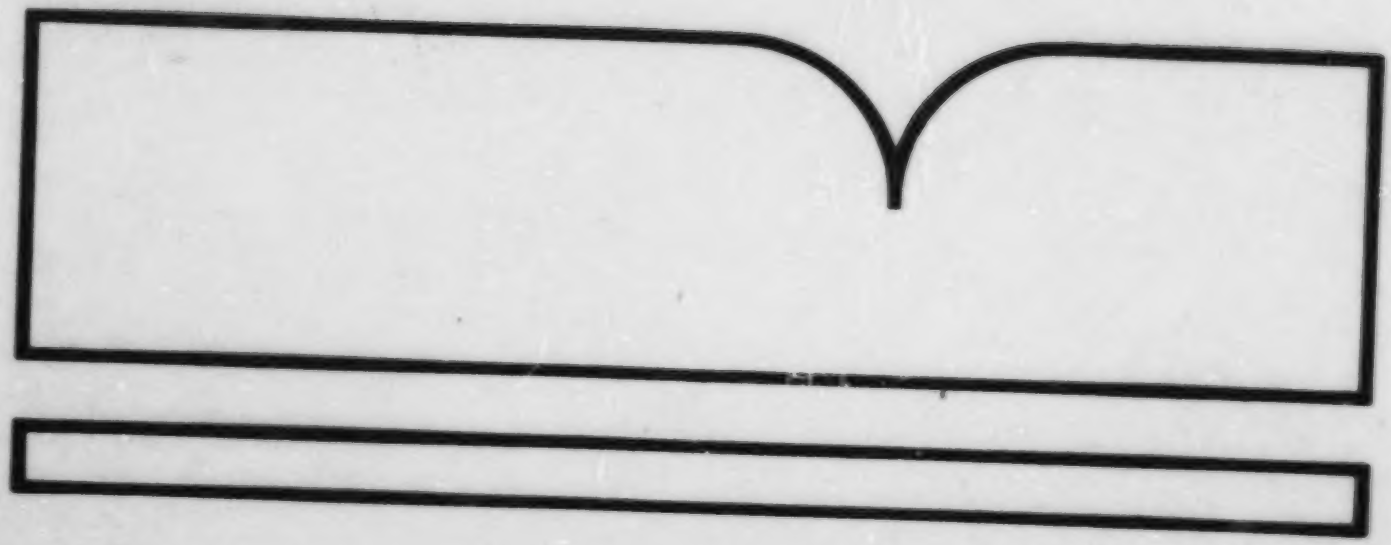

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16. ABSTRACT

Toxicity of Mercuric Chloride to the Developing Rat Kidney. II. Effect of Increased Dosages on Renal Function in Suckling Pups. DASTON, G. P., GRAY, J. A., CARVER, B., AND KAVLOCK, R. J. (1984). *Toxicol. Appl. Pharmacol.* 74, 35–45. It has been demonstrated that the sensitivity to $HgCl_2$ nephrotoxicity increases with maturity in the rat, and that neonates are largely unaffected by a dose of 5 mg/kg. In the present study, immature rat pups were exposed to higher doses of $HgCl_2$ to determine whether this effect was attributable to a quantitative or qualitative difference in the renal sensitivity to $HgCl_2$. Sprague–Dawley rats were injected with a single dose of 5, 7.5, 10, 12.5, 20, or 30 mg/kg on Postnatal Day 1; 5, 7.5, 10, 12.5, 15, or 20 mg/kg on Day 8; or 6.25, 7.5, 10, or 12.5 mg/kg on Day 15. Renal function was evaluated at 24, 48, and 120 hr after treatment by measuring urine volume, osmolality, urinary pH, and chloride content, the ability to concentrate urine during water deprivation, and the presence of protein, glucose, or hemoglobin in urine. Animals were then killed and their kidneys weighed. A dose of 20 mg/kg was needed to induce mortality in pups treated at 1 day of age, and 15 mg/kg was needed in pups treated at 8 days of age. In contrast, the 6.25-mg/kg dose given to 15-day-old pups produced some mortality, and all rats given higher doses at 15 days of age died within 2 days. There was marked oliguria or anuria in the rats that died. Kidney weight was increased in a dose-related fashion at all ages. In those animals not rendered oliguric by the treatment, urine volume increased and the ability to secrete a more concentrated urine during water deprivation decreased. Urinary pH was decreased in a dose-related manner. Urinary chloride excretion was temporarily decreased after $HgCl_2$ treatment on Day 1, but was increased thereafter. Proteinuria, glucosuria, and hematuria were detected in the treated rats, again increasing in frequency and severity with age and dose.

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Toxicity of Mercuric Chloride to the Developing Rat Kidney. II. Effect of Increased Dosages on Renal Function in Suckling Pups

GEORGE P. DASTON,[1] JACQUELINE A. GRAY, BRENDA CARVER, AND ROBERT J. KAVLOCK

Perinatal Toxicology Branch, Developmental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

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Toxicity of Mercuric Chloride to the Developing Rat Kidney. II. Effect of Increased Dosages on Renal Function in Suckling Pups. DASTON, G. P., GRAY, J. A., CARVER, B., AND KAVLOCK, R. J. (1984). *Toxicol. Appl. Pharmacol.* **74**, 35–45. It has been demonstrated that the sensitivity to $HgCl_2$ nephrotoxicity increases with maturity in the rat, and that neonates are largely unaffected by a dose of 5 mg/kg. In the present study, immature rat pups were exposed to higher doses of $HgCl_2$ to determine whether this effect was attributable to a quantitative or qualitative difference in the renal sensitivity to $HgCl_2$. Sprague–Dawley rats were injected with a single dose of 5, 7.5, 10, 12.5, 20, or 30 mg/kg on Postnatal Day 1; 5, 7.5, 10, 12.5, 15, or 20 mg/kg on Day 8; or 6.25, 7.5, 10, or 12.5 mg/kg on Day 15. Renal function was evaluated at 24, 48, and 120 hr after treatment by measuring urine volume, osmolality, urinary pH, and chloride content, the ability to concentrate urine during water deprivation, and the presence of protein, glucose, or hemoglobin in urine. Animals were then killed and their kidneys weighed. A dose of 20 mg/kg was needed to induce mortality in pups treated at 1 day of age, and 15 mg/kg was needed in pups treated at 8 days of age. In contrast, the 6.25-mg/kg dose given to 15-day-old pups produced some mortality, and all rats given higher doses at 15 days of age died within 2 days. There was marked oliguria or anuria in the rats that died. Kidney weight was increased in a dose-related fashion at all ages. In those animals not rendered oliguric by the treatment, urine volume increased and the ability to secrete a more concentrated urine during water deprivation decreased. Urinary pH was decreased in a dose-related manner. Urinary chloride excretion was temporarily decreased after $HgCl_2$ treatment on Day 1, but was increased thereafter. Proteinuria, glucosuria, and hematuria were detected in the treated rats, again increasing in frequency and severity with age and dose.

Mercuric chloride is a potent nephrotoxin in the adult animal. It affects the proximal tubule and may cause acute renal failure, characterized by diuresis or, in extreme cases, oliguria; and elevated values for serum creatinine and urea nitrogen (McDowell *et al.*, 1976). Comparatively little is known, however, of the toxicity of $HgCl_2$ to the postnatally maturing kidney and the reported findings are equivocal. Bidani *et al.* (1979) reported that 2-week-old rats had higher serum urea nitrogen levels and a higher rate of mortality after $HgCl_2$ administration than did rats treated at 4 and 8 weeks of age, suggesting that the young rat is more sensitive to $HgCl_2$ exposure. Wold *et al.* (1977) found no difference in $HgCl_2$-induced uremia between 5-, 15-, and 30-day-old and adult rabbits indicating that there is no age difference in the nephrotoxic response in rabbits. Kavlock (1982) and Kavlock and Gray (1983) in-

[1] George Daston is a National Research Council–Environmental Protection Agency Research Associate. To whom requests for reprints should be addressed. Present address: Department of Zoology, University of Wisconsin, P.O. Box 413, Milwaukee, Wisconsin 53201.

jected rat pups sc on the day of birth with a dose of $HgCl_2$ which is nephrotoxic in the adult rat, but found no alterations in the ability to excrete a water load or to concentrate urine during water deprivation. It appears from these results that the neonate is less sensitive to $HgCl_2$ toxicity. Recently, Daston *et al.* (1983) measured numerous renal functional and morphological parameters in 1-, 8-, 15-, 22-, and 29-day-old rats injected sc with 5 mg/kg $HgCl_2$. For all parameters measured, neonates were found to be practically insensitive to $HgCl_2$ nephrotoxicity, but susceptibility increased rapidly during the first weeks after birth. The most pronounced renal effect was oliguria or anuria. This renal shutdown was observed only in the older age groups and was generally followed by death of the affected individual (Daston *et al.*, 1983).

The relative insensitivity of the immature rat kidney to $HgCl_2$ may be the result of differences in distribution or excretion of Hg. Only 35% of the Hg in an iv dose of $HgCl_2$ is found in the kidneys of 2-week-old rats, as compared with more than 90% to the adult kidney (Jugo, 1975). Thomas and Smith (1979) injected 1-week-old rats with trace amounts of $HgCl_2$ and found that there was no measurable renal excretion of Hg until Postnatal Day 17. Renal Hg levels were also low until Day 17, but increased concomitantly with the onset of Hg excretion. Thus, it may be that the insensitivity of the immature rat kidney to $HgCl_2$ observed previously (Daston *et al.*, 1983) may be due to a decreased renal Hg burden in these young animals. If so, increasing the dosage of $HgCl_2$ to the immature rats may elevate the renal Hg concentration and produce the toxic effects observed in adult rats. It was the purpose of the present study to determine whether higher doses of $HgCl_2$ given to rats from birth to 2 weeks of age would in fact result in comparable toxicity to that reported for older animals, or whether the relative lack of toxicity would persist, suggesting a qualitative difference in immature and mature rats in the interaction of mercury with the nephron.

METHODS

Animals. Time pregnant Sprague–Dawley CD rats[2] were received on Day 15 of gestation (sperm positive day = Day 1). The dams were caged individually on wood shavings in a bio-clean room and fed commercial lab chow[3] and tap water *ad libitum*. Environmental parameters of the room were kept constant (20 to 24°C, 40 to 50% relative humidity, 12-hr light/day).

Animals gave birth on Gestation Day 22. The following day was designated as Postnatal Day 1. All pups were removed from their dams on Day 1 and placed in a common pool. Five male and five female pups were randomly selected from this pool for each dam.

Two studies, with two separate dosing protocols, were carried out. In the first study, pups were given a single sc injection of $HgCl_2$[4] on Day 1, 8, or 15. The Day 1 and Day 8 pups received doses of 0, 7.5, and 12.5 mg/kg. Fifteen-day-old rats were far more susceptible to $HgCl_2$ toxicity; hence, they received 6.25, 7.5, 10, and 12.5 mg/kg. The dosing solutions were made up in 0.85% NaCl to concentrations which gave injection volumes of 3.3 μl/g body wt. These dosages of $HgCl_2$ were insufficient to induce oliguria in the two youngest age groups; hence, a second study with higher doses was undertaken. In this study, 1- and 8-day-old pups were injected with higher doses of $HgCl_2$ in an attempt to define the dose at which animals of this age develop renal shutdown as seen in adults. In this study, Day 1 pups received a sc injection of 0, 5, 10, 20, or 30 mg/kg $HgCl_2$. Day 8 pups received 0, 5, 10, 15, or 20 mg/kg $HgCl_2$.

Renal function assessments. Renal function was evaluated 24, 48, and 120 hr postinjection (pi). Pups were removed from their home cage and voided of urine. All urine collection was accomplished by perineal stimulation, which induces micturition in suckling rats (Kavlock and Gray, 1982). A urine sample from the initial voiding was assessed for osmotic content by a vapor pressure osmometer.[5] The pups were maintained in beakers containing wood shavings in a 35°C water bath for 4 hr. Urine was collected after this period of fluid deprivation and its osmolality was measured. Urinary chloride content was measured coulometrically.[6] Urinary pH was determined with a micro-combination probe.[7] The presence of protein,

[2] Charles River Breeding Laboratories, Wilmington, Mass.

[3] Purina Laboratory Chow.

[4] Aldrich Chemical Co., Milwaukee, Wisc., 99.9% pure. This was stored in a desiccator at 22°C, and is stable indefinitely under these conditions. Dosing solutions were prepared the day before use.

[5] Wescor Model 5100B, Logan, Utah.

[6] Radiometer Copenhagen CMT-10 Chloride Titrator, Copenhagen, Denmark.

[7] Microelectrodes, Inc., Londonderry, N.H.

glucose, and blood in the urine was detected qualitatively in 8- and 15-day-old pups with clinical reagent strips in a semiautomated photoanalyzer.[8]

Following the 4-hr hydropenia period, the animals were killed by decapitation and the kidneys were weighed.

Statistics. Lower dosages caused diuresis, but higher dosages induced severe oliguria. (We defined oliguria as a urine volume which was at least two standard deviations lower than the mean control urine volume.) The amount of urine collected from oliguric rats was insufficient for measurement of functional parameters; hence, the data from these animals were analyzed separately. Probit analysis (Finney, 1971) was used to calculate the LD50 and the ED50 for oliguria, along with upper and lower 95% confidence limits, for each age group. These data are presented in Table 1.

Data from rats which were not oliguric were analyzed by the general linear models procedure available on the Statistical Analysis System (Helwig and Council, 1979). These data are presented in Tables 2 through 6. Two-way analysis of variance was employed to test for interaction between dosage of $HgCl_2$ and time after dosing for each parameter within each age group. When there was no significant interaction, the data from all three postdosing observation periods were pooled within each dose group. Otherwise, each observation period was considered separately. Differences between treated and control groups were analyzed by comparing least square means. Dose-related phenomena were detected by analysis of variance. All data were reported as mean ± standard error of the mean.

RESULTS

Oliguria and Mortality

The most pronounced form of renal toxicity observed after $HgCl_2$ treatment was renal shutdown leading to severe oliguria or anuria. Oliguria was most frequent 24 hr pi, and was almost always followed by death of the affected individual. The dose of $HgCl_2$ needed to produce oliguria decreased with postnatal age. A dose of 30 mg/kg caused 80% of the pups treated on Day 1 to be oliguric 24 hr pi; 20 mg/kg on Day 8 caused 87.5% to be oliguric; and 7.5 mg/kg on Day 15 caused 100% of the pups to be oliguric at 24 hr pi (Table 1). The ED50 for producing oliguria at each age is presented in Table 1, along with upper and lower 95% confidence limits. None of the confidence intervals for the three age groups overlap, providing statistical evidence that the ED50 of $HgCl_2$ for renal shutdown decreases in each succeeding age group.

Almost all of the rats which were oliguric 24 hr pi died by the end of the observation period, and the LD50 and ED50 for oliguria are similar within each age group (Table 1). The LD50 for rats treated on Day 8 could not be calculated from the data; however, based on the available data it would probably fall between 15 and 20 mg/kg, roughly equivalent to the oliguria ED50 at that age.

Renal Function

The animals which were not oliguric were assessed for renal function. These results are grouped by age and are presented below.

Day 1 exposure. Neonatal rats were the least affected by $HgCl_2$ exposure. The highest dose used in the first study, 12.5 mg/kg, was insufficient to produce oliguria in Day 1 rats. Therefore, a second dose–response study, with doses as high as 30 mg/kg, was conducted. The data from the first and second studies are presented in Tables 2 and 3, respectively.

A dose of 20 mg/kg $HgCl_2$ on Day 1 caused oliguria in 20% of the pups, and 30 mg/kg induced renal shutdown in 80% and death in 95% (Table 1). Therefore, no renal function data were obtained from animals given 30 mg/kg $HgCl_2$.

Body weight was decreased in the 10- and 20-mg/kg groups 120 hr pi (Table 3). Kidney/body wt ratio was increased 120 hr pi by doses of 7.5 mg/kg and higher (Tables 2, 3). This effect was significantly dose related ($p < 0.001$). Elevated kidney weight is a common finding in acute renal failure and is probably due to interstitial edema.

Basal urinary osmolality was increased above the control level 48 and 120 hr pi in the 10- and 20-mg/kg dose groups (Table 3). In the first study, urine volumes were significantly increased above the control value in the 7.5- and 12.5-mg/kg dose groups 48 hr pi,

[8] Clini-Tek, Ames Co., Elkhart, Ind.

TABLE 1

MORTALITY AND OLIGURIA INDUCED BY $HgCl_2$ INJECTION IN IMMATURE RATS

	$HgCl_2$ (mg/kg)	n	% Dying by 120 hr pi	% Oliguric by 24 hr pi	LD_{50}[a]	Upper and lower limits of LD_{50} (at 0.95)[a]	ED_{50} for oliguria[a]	Upper and lower limits of ED_{50} (at 0.95)[a]
Day 1					20.3	17.1–23.4	24.5	21.6–27.8
	0	19	0	0				
	5	20	0	0				
	10	20	5.0	0				
	20	20	35.0	20.0				
	30	20	95.0	80.0				
Day 8					[b]	[b]	17.5	16.3–18.8
	0	19	0	0				
	5	13	0	0				
	10	19	0	0				
	15	20	0	10.0				
	20	16	56.2	87.5				
Day 15					5.9	5.6–6.1	6.1[c]	5.8–6.4[c]
	0	30	0	0				
	5[d]	38	10.5	13.2				
	6.25	30	70.0	43.3				
	7.5	30	96.7	100.0				
	10	30	100.0	100.0				
	12.5	39	100.0	100.0				

[a] Calculated by probit analysis.
[b] Not calculable from the data.
[c] The dose–response curve for the oliguria data for Day 15 rats was significantly nonlinear; therefore, these values are only approximate.
[d] The data for this dose level are from a previous study (Daston *et al.*, 1983).

and in the 12.5-mg/kg group 120 hr pi (Table 2). Diuresis was observed 120 hr pi in the 10- and 20-mg/kg groups in the second study (Table 3). The polyuria in both studies was significantly dose related. The ability to concentrate solutes in response to water deprivation was only impaired at 12.5 mg/kg in study 1 (Table 2).

Urinary chloride excretion is normally high on the first day after birth, then decreases steadily over the first postnatal week and remains low until weaning. Urinary chloride excretion was significantly decreased 24 hr after treatment with 12.5 (Table 2) and 20 (Table 3) mg/kg $HgCl_2$. Thereafter, chloride excretion was increased by the $HgCl_2$ treatment. This increase was statistically significant in the 5-, 12.5-, and 20-mg/kg groups 48 hr pi, and in the 20-mg/kg group 120 hr pi (Tables 2, 3).

A decrease in urinary pH was observed 48 and 120 hr pi at all dose levels above 5 mg/kg (Tables 2, 3). This was a significantly dose-related phenomenon ($p < 0.002$) at both observation times in both studies. There was a significant increase in urine pH 24 hr pi in the 10- and 20-mg/kg groups (Table 3). There was also an increase in pH in the 12.5-mg/kg group at this time, but this was not statistically significant.

Day 8 exposure. Rats treated at 8 days of age were more sensitive than neonates to $HgCl_2$ nephrotoxicity. In the first study, the 12.5-mg/kg dose was fatal to one rat and

TABLE 2
EFFECTS OF $HgCl_2$ INJECTION ON DAY 1 ON RENAL PARAMETERS—STUDY 1

$HgCl_2$ dose (mg/kg)	Hours postinjection	n	Body weight[a] (g)	Kidney wt/ body wt (%)	Basal urinary osmolality[a] (mOsM)	Urine osmolality after 4-hr water deprivation[a] (mOsM)	Relative urine volume (μl/hr/g body wt)	Urinary pH	Urinary chloride excretion (nEq/g body wt/hr)
0	24	6	5.9 ± 0.4	1.09 ± 0.04	322 ± 19	415 ± 19	2.37 ± 0.28	5.94 ± 0.24	151 ± 37
	48	7	7.0 ± 0.5	1.29 ± 0.07	331 ± 61	496 ± 33	2.56 ± 0.49	5.97 ± 0.17	112 ± 27
	120	5	11.2 ± 1.1	1.32 ± 0.07	369 ± 89	664 ± 118	2.11 ± 0.47	6.37 ± 0.29	84 ± 14
7.5	24	7	5.9 ± 0.3	1.31 ± 0.06	239 ± 21	332 ± 27	3.47 ± 0.64	5.47 ± 0.29	103 ± 14
	48	7	7.4 ± 0.2	1.31 ± 0.05	351 ± 25	417 ± 28	5.00 ± 0.50[b]	5.34 ± 0.11[b]	180 ± 28
	120	6	10.5 ± 0.8	1.52 ± 0.05[b]	428 ± 77	663 ± 72	2.30 ± 0.18	5.53 ± 0.12[b]	97 ± 19
12.5	24	11	6.1 ± 0.2	1.31 ± 0.03	248 ± 19	266 ± 20[b]	2.99 ± 0.23	6.18 ± 0.23	71 ± 13[b]
	48	10	7.1 ± 0.3	1.41 ± 0.04	312 ± 11	379 ± 21[b]	4.28 ± 0.36[b]	5.05 ± 0.09[b]	203 ± 35[b]
	120	9	10.8 ± 0.4	1.41 ± 0.04	454 ± 17	530 ± 21[b]	3.44 ± 0.13[b]	4.95 ± 0.05[b]	136 ± 21

[a] In making statistical comparisons, observations for this parameter were pooled for all three observation times within each dose group, since there was no significant interaction between dose of $HgCl_2$ and hours postinjection.
[b] Significantly different from controls by general linear models procedure, $p < 0.05$.

caused anuria in two others 120 hr pi. The second dose–response study employed 20 mg/kg as the high dose. This dose caused mortality in more than half of the treated pups, and oliguria in 87.5%. Data from the two dose–response studies are presented in Tables 4 and 5.

Body weight was 16 to 46% lower than the control value in all dose groups 120 hr pi (Tables 4, 5). Decreased body weight was significantly dose related ($p < 0.004$) in both studies. Kidney/body wt ratio was increased at 120 hr pi in all dose groups (Tables 4, 5) again in a dose-related fashion. Kidney/body wt ratio was also increased 24 hr pi by the 12.5-mg/kg dose, and at 48 hr pi by both doses in study 1 (Table 4). Kidney weight was not determined at these times in study 2.

Urine volume was increased by the $HgCl_2$ treatment. In study 1, both doses caused a significant dose-related ($p < 0.001$) diuresis 24 and 48 hr pi (Table 4). In study 2, urine volume was increased in the 5-mg/kg group 24 hr pi, and in all dose groups 48 and 120 hr pi (Table 5). Urine volume was positively correlated with dose of $HgCl_2$ ($p < 0.005$) at all observation times in study 2.

Basal urinary osmolality was decreased 24 hr pi by all dose groups in study 2 (Table 5). Subsequently, basal osmolality was increased 48 hr ip by the 7.5- and 12.5-mg/kg $HgCl_2$ doses in study 1 (Table 4) and 120 hr pi by all the $HgCl_2$ dose groups in study 2 (Table 5). Even with the increased basal osmolality, the urinary osmolality after a 4-hr period of water deprivation was significantly lower than controls in all dose groups at all observation times, with the exception of the 7.5-mg/kg group 120 hr pi (Tables 4, 5). This finding indicates a significant deficit in the ability to concentrate solutes in response to fluid deprivation. The decrease in urine osmolality in hydropenic rats was dose related ($p < 0.03$) at all observation times.

Urinary excretion of chloride was increased by $HgCl_2$ treatment on Day 8. This increase was observed 24 hr pi in the 7.5-mg/kg group, 24 and 48 hr pi in the 12.5-mg/kg group of

TABLE 3

EFFECTS OF $HgCl_2$ INJECTION ON DAY 1 ON RENAL PARAMETERS—STUDY 2[a]

$HgCl_2$ (mg/kg)	Hours postinjection	n	Body wt (g)	Kidney wt/ body/wt (%)	Basal urine osmolality (mOsM)	Urine osmolality after 4-hr hydropenia (mOsM)[b]	Relative urine volume (μl/g body wt/hr)	Urine pH	Urinary chloride excretion (nEq/g body wt/hr)
0	24	9	6.0 ± 0.2	ND[c]	260 ± 38	328 ± 19	2.63 ± 0.33	5.31 ± 0.06	170 ± 20
	48	9	6.2 ± 0.2	ND	247 ± 28	400 ± 36	2.20 ± 0.18	5.50 ± 0.11	134 ± 8
	120	10	10.4 ± 0.3	1.47 ± 0.03	210 ± 19	394 ± 21	2.76 ± 0.29	6.24 ± 0.12	82 ± 10
5	24	10	6.0 ± 0.3	ND	322 ± 33	334 ± 25	2.48 ± 0.32	5.51 ± 0.19	124 ± 8
	48	10	6.9 ± 0.2	ND	271 ± 20	423 ± 11	2.88 ± 0.26	5.45 ± 0.15	196 ± 30[d]
	120	10	9.9 ± 0.3	1.47 ± 0.03	284 ± 22	465 ± 21	2.80 ± 0.23	5.99 ± 0.09	86 ± 14
10	24	10	5.6 ± 0.2	ND	262 ± 21	313 ± 9	2.67 ± 0.31	6.86 ± 0.37[d]	171 ± 40
	48	10	5.6 ± 0.3	ND	331 ± 13[d]	385 ± 12[d]	2.68 ± 0.20	5.04 ± 0.15[d]	174 ± 25
	120	10	7.6 ± 0.6[c]	1.65 ± 0.07[d]	347 ± 67[d]	454 ± 32	2.89 ± 0.25[d]	5.21 ± 0.09[d]	116 ± 14
20	24	8	5.5 ± 0.2	ND	226 ± 17	281 ± 10	1.74 ± 0.29	6.88 ± 0.13[d]	60 ± 19[d]
	48	9	5.5 ± 0.2	ND	383 ± 14[d]	372 ± 13	2.86 ± 0.21	4.79 ± 0.07[d]	204 ± 24[d]
	120	9	8.4 ± 0.6[c]	1.65 ± 0.05[d]	389 ± 30[d]	432 ± 16	3.19 ± 0.13[d]	5.12 ± 0.05[d]	138 ± 14[d]

[a] Data from rats treated with 30 mg/kg were not included in this table, since these animals were oliguric by 24 hr postinjection.

[b] In making statistical comparisons, observations for this parameter were pooled for all three observation times within each dose group, since there was no significant interaction between dose of $HgCl_2$ and hours postinjection.

[c] ND = not determined.

[d] Significantly different from controls by general linear models procedure, $p < 0.05$.

TABLE 4

EFFECTS OF $HgCl_2$ INJECTION ON DAY 8 ON RENAL PARAMETERS—STUDY 1

$HgCl_2$ (mg/kg)	Hours postinjection	n	Body wt (g)	Kidney wt/body wt (%)	Basal urinary osmolality (mOsM)	Urine osmolality after 4-hr water deprivation (mOsM)	Relative urine volume (μl/hr/g body wt)	Urinary pH[a]	Urinary chloride excretion (nEq/g body wt/hr)	Proteinuria (% individuals affected)	Glucosuria (% individuals affected)	Hematuria (% individuals affected)
0	24	6	15.5 ± 0.5	1.34 ± 0.03	214 ± 13	584 ± 25	2.03 ± 0.28	6.43 ± 0.16	35 ± 4	0	0	16.7
	48	7	18.3 ± 1.3	1.40 ± 0.01	231 ± 27	693 ± 22	2.21 ± 0.15	6.13 ± 0.12	28 ± 4	0	0	
	120	7	27.3 ± 1.3	1.41 ± 0.02	571 ± 48	726 ± 46	3.51 ± 0.35	5.41 ± 0.15	74 ± 18	14.1	0	0
7.5	24	9	16.0 ± 1.0	1.47 ± 0.04	191 ± 9	257 ± 19[b]	4.47 ± 0.37[b]	5.40 ± 0.16[b]	109 ± 21[b]	16.7	0	55.6
	48	10	18.3 ± 0.8	1.80 ± 0.07[b]	299 ± 10[b]	387 ± 11[b]	4.99 ± 0.16[b]	5.17 ± 0.06[b]	73 ± 10	40.0	0	0
	120	11	1?.2 ± 1.3[b]	2.15 ± 0.14[b]	543 ± 34	645 ± 46	3.27 ± 0.43	4.73 ± 0.05[b]	69 ± 27	100.0	0	18.2
12.5	24	9	16.0 ± 0.7	1.60 ± 0.05[b]	218 ± 13	274 ± 16[b]	4.29 ± 0.69[b]	5.68 ± 0.12[b]	106 ± 24[b]	100.0	33.3	77.0
	48	10	18.4 ± 1.3	2.00 ± 0.07[b]	348 ± 10[b]	440 ± 17[b]	5.07 ± 0.42[b]	5.23 ± 0.66[b]	134 ± 18[b]	80.0	10.0	10.0
	120	8	14.7 ± 0.6[b]	2.53 ± 0.09[b]	541 ± 11	569 ± 53[b]	2.26 ± 0.75	4.86 ± 0.14[b]	45 ± 18	87.5	0	62.0

[a] In making statistical comparisons, observations for this parameter were pooled for all observation times within each dose group, since there was no significant interaction between dose of $HgCl_2$ and hours postinjection.

[b] Significantly different from controls by general linear models procedure, $p < 0.05$.

study 1 (Table 4), and 48 hr pi in all the dose groups of study 2 (Table 5).

Urinary pH was lowered by as much as a log unit at all observation times in both dose groups of study 1 (Table 4). This effect was also observed in study 2, with the 5- and 10-mg/kg groups having more acidic urine 24 hr pi, the 5, 10, and 15 mg/kg groups having more acidic urine 48 hr pi, and all dose groups having more acidic urine 120 hr pi (Table 5). Although the higher dose levels required a longer period of time to cause significant acidification of the urine, the degree of acidification was significantly dose related ($p < 0.001$) by 120 hr pi.

Proteinuria, glucosuria, and hematuria were observed after $HgCl_2$ injection on Day 8. The incidence of these conditions also increased with dose.

Day 15 exposure. Of the three age groups examined, Day 15 rats were the most susceptible to $HgCl_2$-induced nephrotoxicity. Doses of 10 and 12.5 mg/kg caused 100% mortality in all rats exposed when 15 days of age. By 24 hr pi, 11% of the pups dosed with 7.5 mg/kg had died, and the remaining rats were oliguric. Of the 30 animals in this group, 29 died by 120 hr pi. The 6.25-mg/kg dose of $HgCl_2$ caused no deaths 24 hr pi, but 43.3% of the pups were oliguric. By 48 hr pi, 33% of the pups in this group died, and 70% were dead by 120 hr pi. Since 6.25 mg/kg was the only dose of $HgCl_2$ which did not cause renal shutdown 100% of the time, renal function assessments were made for this group only on Day 15.

Kidney/body wt ratio in the 6.25-mg/kg group was significantly increased 48 and 120 hr pi (Table 6). There was a significant diuresis in the treated group 24 and 48 hr pi. The urine volume of the 6.25-mg/kg group was more than twice the control value 48 hr pi (Table 6). Basal urinary osmolality was decreased by the 6.25-mg/kg dose 48 and 120 hr pi. The ability to produce a concentrated urine during fluid deprivation was compromised by the $HgCl_2$ treatment at all observation times (Table 6). Urinary chloride excretion in the treated

TABLE 5

EFFECTS OF $HgCl_2$ INJECTION ON DAY 8 ON RENAL PARAMETERS—STUDY 2[a]

$HgCl_2$ (mg/kg)	Hours postinjection	n	Body wt (g)	Kidney wt/ body wt (%)	Basal urine osmolality (mOsM)	Urine osmolality after 4-hr hydropenia (mOsM)[b]	Relative urine volume (μl/g body wt/hr)	Urine pH	Urinary chloride excretion (nEq/g body wt/hr)
0	24	10	15.4 ± 0.8	ND[c]	338 ± 15	546 ± 44	2.07 ± 0.12	6.43 ± 0.15	56 ± 6
	48	9	17.4 ± 0.8	ND	324 ± 27	526 ± 47	2.19 ± 0.27	6.31 ± 0.15	52 ± 15
	120	10	23.5 ± 1.1	1.39 ± 0.03	283 ± 31	588 ± 65	1.76 ± 0.12	6.35 ± 0.16	44 ± 8
5	24	8	14.8 ± 0.7	ND	238 ± 12[d]	284 ± 25[d]	3.42 ± 0.36[d]	5.84 ± 0.13[d]	87 ± 16
	48	8	15.5 ± 0.7	ND	350 ± 23	386 ± 25[d]	3.63 ± 0.34[d]	5.16 ± 0.07[d]	93 ± 12[d]
	120	8	19.9 ± 0.9[d]	1.68 ± 0.09[d]	432 ± 84[d]	462 ± 68[d]	3.05 ± 0.31[d]	5.10 ± 0.10[d]	43 ± 7
10	24	10	16.5 ± 0.5	ND	232 ± 17[d]	215 ± 12[d]	2.66 ± 0.25	5.69 ± 0.12[d]	42 ± 6
	48	9	15.9 ± 0.8	ND	326 ± 7	346 ± 7[d]	4.94 ± 0.23[d]	4.99 ± 0.06[d]	121 ± 18[d]
	120	10	19.5 ± 1.0[d]	1.73 ± 0.08[d]	391 ± 11[d]	378 ± 13[d]	4.42 ± 0.24[d]	4.89 ± 0.07[d]	90 ± 7[d]
15	24	9	15.9 ± 0.6	ND	237 ± 9[d]	235 ± 11[d]	2.35 ± 0.24	6.28 ± 0.20	31 ± 5
	48	10	16.4 ± 0.4	ND	325 ± 6	354 ± 13[d]	4.50 ± 0.57[d]	5.19 ± 0.10[d]	85 ± 21[d]
	120	10	18.5 ± 1.2[d]	2.14 ± 0.14[d]	418 ± 20[d]	448 ± 29[d]	4.27 ± 0.54[d]	4.72 ± 0.10[d]	38 ± 9
20	48	5	15.0 ± 1.0	ND	422 ± 70[d]	403 ± 102[d]	7.23 ± 2.17[d]	5.91 ± 0.45	150 ± 41[d]
	120	3	15.8 ± 2.3[d]	2.18 ± 0.25[d]	436 ± 15[d]	417 ± 15[d]	2.88 ± 0.69	4.64 ± 0.07[d]	53 ± 18

[a] Data for the 24-hr pi period from rats treated with 20 mg/kg were not included in this table, since all but two of these animals were oliguric at this time.

[b] In making statistical comparisons, observations for this parameter were pooled for all three observation times within each dose group, since there was no significant interaction between dose of $HgCl_2$ and hours postinjection.

[c] ND = not determined.

[d] Significantly different from controls by general linear models procedure, $p < 0.05$.

group was more than four times that of the control 48 hr pi ($p < 0.05$). Urinary pH was significantly lowered by the $HgCl_2$ treatment (Table 6). Abnormal urine constituents—protein, glucose, and hemoglobin—were detected in rats treated with 6.25 mg/kg $HgCl_2$ on Day 15 (Table 6).

DISCUSSION

It has been demonstrated previously that a single sc injection of 5 mg/kg $HgCl_2$ induced renal toxicity, the severity being correlated with the stage of postnatal maturation of the animal (Daston *et al.*, 1983). The present study demonstrated that at each age, the effects produced by $HgCl_2$ were dose related and that the dosage required to produce severe toxicity decreased as the rats matured. After neonatal exposure, relative kidney weights, hydropenic urinary osmolality, urine volume, urinary pH, and urinary chloride excretion were affected when the dosage of $HgCl_2$ was sufficiently high. In addition to these parameters, body weight and basal urine osmolality were affected on Day 8, and pathological components—protein, glucose, and occult blood—were present in the urine of treated pups. Treatment-related effects were most severe in Day 15 rats, and mortality was high at the dosages of $HgCl_2$ used.

The findings of the present study indicate that there is no qualitative difference among rats that are 1, 8, or 15 days of age and that reported for adult rats (McDowell *et al.*, 1976) in their renal response to $HgCl_2$. When sufficiently high dosages of $HgCl_2$ were given these animals, they developed all of the signs of acute renal failure that are observed in adults after exposure. Severe oliguria, anuria, and death were observed at sufficiently high doses. Kidney weight was increased, renal functional capacity was decreased, and abnormal urinary constituents indicating severe renal damage were frequently detected. As with less severe manifestations of nephrotoxicity, the neonate required the highest dosage of $HgCl_2$ to precipitate renal shutdown. Sen-

TABLE 6

EFFECTS OF $HgCl_2$ INJECTION ON DAY 15 ON RENAL PARAMETERS[a]

$HgCl_2$ (mg/kg)	Hours postinjection	n	Body weight (g)	Kidney wt/body wt (%)	Basal urine osmolality (mOsM)	Urine osmolality after 4-hr hydropenia (mOsM)[b]	Relative urine volume (μl/g body wt/hr)	Urine pH[b]	Urinary chloride excretion (nEq/g body wt/hr)	Proteinuria (% individuals affected)	Glucosuria (% individuals affected)	Hematuria (% individuals affected)
0	24	9	32.3 ± 1.1	1.21 ± 0.02	330 ± 44	730 ± 65	2.05 ± 0.33	6.15 ± 0.09	34 ± 11	0	0	0
	48	10	33.2 ± 1.1	1.30 ± 0.02	460 ± 50	756 ± 52	2.45 ± 0.31	6.18 ± 0.07	46 ± 17	0	0	0
	120	12	44.2 ± 0.9	1.23 ± 0.02	535 ± 67	1221 ± 81	1.42 ± 0.24	6.05 ± 0.05	104 ± 21	16.7	8.3	0
6.25	24	5	31.9 ± 0.8	1.46 ± 0.06	326 ± 47	372 ± 80[c]	3.43 ± 0.70[c]	5.71 ± 0.17[c]	51 ± 19	60.0	40.0	0
	48	6	35.9 ± 0.6	1.94 ± 0.05[c]	307 ± 26[c]	441 ± 52[c]	5.85 ± 1.07[c]	5.62 ± 0.41[c]	235 ± 37[c]	50.0	50.0	33.3
	120	4	41.0 ± 3.0	3.19 ± 0.58[c]	366 ± 85[c]	645 ± 35[c]	2.26 ± 0.41	5.56 ± 0.23[c]	90 ± 27	75.0	25.0	0

[a] Data from rats treated with 7.5 mg/kg $HgCl_2$ at this age are not included, since all of these animals were oliguric by 24 hr pi.
[b] In making statistical comparisons, observations for this parameter were pooled for all three observation times within each dose group, since there was no significant interaction between dose of $HgCl_2$ and hours postinjection.
[c] Significantly different from controls using general linear models procedure, $p < 0.05$.

sitivity to the $HgCl_2$-induced nephrotoxicity increased with age.

Previously published reports on the distribution in Hg in the immature and mature rat indicate that renal Hg accumulation following $HgCl_2$ injection increases with postnatal age. Jugo (1975) reported that 2-week-old rats accumulated only 35% of the Hg from an iv injection of $^{203}HgCl_2$ in the kidney, whereas adults accumulated more than 90%. Similarly, Thomas and Smith (1979) found that fractional distribution of Hg to the rat kidney increased during postnatal development. They observed that after sc injection of $HgCl_2$ on Day 7, only 20% of the total body burden of Hg was found in the kidney on Day 8, increasing to almost 40% by Day 17 and 70% by Day 28. Thus, the injection of equivalent dosages of $HgCl_2$ does not result in equivalent renal Hg concentrations in rats of different ages. Higher dosages of $HgCl_2$ would be needed in subadult rats to produce a renal Hg concentration (and ostensibly a similar toxic response) comparable to that in adults. One possibility for the decreased distribution of Hg to the young rat kidney is that glomerular filtration rate is low in the neonate, but increases during the first weeks after birth (Horster and Lewy, 1970). Thus, less Hg would be present in the lumens of the tubules, perhaps resulting in less toxicity to the tubular epithelium.

Decreased renal Hg concentrations in immature rats may be due to sequestering of the metal in another organ. Divalent metals such as Hg are bound by the protein metallothionein (Kagi *et al.*, 1974), providing protection against toxicity. Metallothionein is present in extremely low concentrations in mature rats which have not been previously exposed to metals; however, hepatic metallothionein concentration has been reported to be 8- to 20-fold (Bell, 1979, 1980; Oh and Whanger, 1979; Wong and Klaassen, 1980) higher in perinatal rats than in postweaning animals, with the levels of this protein constantly diminishing during postnatal maturation (Bell, 1979). This hepatic metallothionein store in immature rats may sequester Hg and protect the kidney from toxicity by decreasing the amount of Hg which would be distributed to it. The nephrotoxicity of $HgCl_2$, which was enhanced as the rats matured (Daston *et al.*, 1983) occurs at the same time as the postnatal decrease in hepatic metallothionein content. Furthermore, the age-related decrease in the amount of $HgCl_2$ needed to produce acute renal failure seen in the present study would also be an expected consequence of constantly decreasing metallothionein stores. However, recent evidence suggests that the metallothionein of young rats may not be functionally equivalent to that of mature animals. Wong and Klaassen (1980) and Bell (1980) have demonstrated that rat pups during the first week after birth have only a limited advantage over postweaning rats in their ability to accumulate cadmium in their liver. Thus, although it is an attractive hypothesis, it is unclear whether the hepatic metallothionein store of immature rats is responsible for their lowered sensitivity to $HgCl_2$ toxicity.

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